Original Article

Pathogenic Role of Interlukin-23(IL-23) in Rheumatoid Arthritis Patients; its Relation to Disease Activity

Doaa S.E. Zaky[1], Zakia A.Z. Mohamed[2], Sabah E. Abd-Elraheem[2], Eslam M.A. El-Nahrery[3]

Departments of Internal Medicine[1], Clinical Pathology[2], Al-Azhar University;
Chemistry[3] (Biochemistry Branch), Faculty of Science, Suez University; Egypt

ABSTRACT

Background: IL-23 is a pro-inflammatory cytokine belonging to the IL-12 cytokine family. IL-23 is essential for the differentiation of T helper 17 (Th17) lymphocytes, a subtype of T lymphocyte implicated in chronic inflammatory/ autoimmune mediated diseases. Experimental models of arthritis and clinical indications have highlighted an important role for Th17 lymphocytes in the pathogenesis of rheumatoid arthritis (RA). However the role and mechanism of action of IL23 in the pathogenesis of RA are still not fully understood. **Objective:** This study was conducted to determine the serum concentration of IL-23 in patients with RA as well as the relationship between the IL-23 level and disease activity. **Methods:** The study included 35 patients with RA fulfilling the American College of Rheumatology (ACR) revised criteria for diagnosis of RA as well as 15 age and sex matched healthy subjects as controls. The clinical parameters of disease activity were determined by the 28-joint disease activity score (DAS-28). Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), rheumatoid factor and Anti-citrullinated protein antibodies (ACPA) were done. The levels of IL-23 were determined by enzyme-linked immunosorbent assay (ELISA). **Results:** Serum level of IL-23 was significantly elevated in RA patients [43.6 (27.8-248.9)] compared to control group [32.1 (27.3-34.9)] ($P < 0.05$). However, no correlations were found between IL-23 and DAS-28 score, ESR or ACPA in RA patients. **Conclusion:** Our results imply that IL-23 may potentially play a role in the pathogenesis of RA and may be a useful index for the diagnosis of this disease. Targeting the IL 23 cytokine may provide a new therapeutic approach in the treatment of RA. **[Egypt J Rheumatology & Clinical Immunology, 2014; 2(1): 63-69]**

Key Words: IL-23, Rheumatoid arthritis, DAS-28 score.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease predominantly manifests as polyarthritis with extra-articular complications. Articular affection in RA is characterized by progressively destructive joint inflammation, destruction of articular cartilage with bone and synovial hyperplasia. Various cell types are involved in the pathogenesis of RA including T cells, antigen presenting cells, and endothelial cells.[1] Cytokines also play a fundamental role in the processes that cause inflammation, articular destruction and extra-articular manifestation associated with RA. IL-23 is a pro-inflammatory cytokine belonging to the IL-12 cytokine family, secreted by activated dendritic cells (DCs) and macrophages. IL-23 binds to an IL-23 receptor expressed on dendritic cells, macrophages and monocytes.[2] IL-23 is essential for the differentiation of Th17 lymphocytes from naive CD4+ T cells.[3] Th17 cells have been associated with the induction of autoimmune tissue inflammation and under the influence of IL-23 produce interleukin-17 (IL-17) and some other additional novel factors.[4] Recent reports have suggested that IL-17-producing Th17 cells are a new subset of cells critical to the pathogenesis of RA. IL-17 induces the production of inflammatory cytokines such as IL-1, IL-6, IL-8, and tumour necrosis factor-α (TNF-α), and it has been detected in the serum, synovial fluid (SF), and synovium of patients with RA.[5] Th17 lymphocytes, when stimulated by IL-23 it promotes osteoclastogenesis inducing receptor activator for Nuclear Factor-k B Ligand (RANKL) on mesenchymal cells and in cultures of osteoblasts. RANKL involved in the regulation of osteoclastogenesis is a key factor of the bone erosion process, and stimulates endothelial cells, epithelial cells, and synovial fibroblasts to produce prostaglandin E2, IL-6, and IL-8.[6] Van Bezooijen et al. and Lubberts et al. found that in patients with RA, IL-17 is involved in the destruction of the extracellular matrix and juxtaarticular bone resorption, through the induction of synthesis of RANKL and matrix metalloproteases.[7,8] Also Stamp et al. demonstrated that IL-23 gene expression is higher in IL-17A+ versus IL-17A- membranes.[9] In keeping with

Correspondence to Doaa Salah-Eldeen Zaky, Department of Internal Medicine, Al Azhar University; Egypt
E-mail: dsalah241@gmail.com

this, IL-17A+ and IL-23+ cells localize in synovial membranes. So in RA, IL23 is an important determinant of the production of IL17A, a cytokine of consequence in inflammation and bone destruction.[10]

This study was designed to characterize the IL 23 role in the pathogenesis of RA and to examine the relationship between IL23 and markers of activity in those patients, helping to identify novel diagnostic and/or therapeutic targets for arthritis in patients with RA.

PATIENTS AND METHODS

Study Population

This study was conducted in Al-Zahraa Hospital of Al-Azhar University. The study population consisted of 35 patients with RA (the patients fulfilling the American College of Rheumatology (ACR) revised criteria for diagnosis of RA[11] as well as 15 age and sex matched healthy volunteers served as control. Patients were selected from the department of Internal Medicine. They were eligible for the study if they were 20 years of age or older. All patients included in the study underwent a standard procedure consisting of detailed medical history as well as physical examination including musculoskeletal system. Body mass index (BMI) was calculated using the equation BMI = weight (in kilograms) divided by the square of the height (in meters). Patients with other autoimmune inflammatory disorders that affect IL-23 level such as ankylosing spondylitis, psoriasis, multiple sclerosis, sarcoidosis and inflammatory bowel diseases were excluded from the study. Disease activity was assessed using the 28-joint disease activity score (DAS-28).[12] The patients were classified according DAS-28 score to: Group 1: include 10 patients in remission (DAS-28 score < 2.6), Group 2: include 8 patients with low disease activity (DAS-28 score ranges between 2.6-3.2), Group 3: include 10 patients with moderate disease activity (DAS-28 score ranges between 3.2-5.1) and Group 4: include 7 patients with High disease activity (DAS-28 score > 5.1). Those who voluntarily decided to participate in the study were signed an informed consent.

Laboratory Studies

Blood samples were taken from patients and control subjects. Each blood sample was divided into two portions as follows: First portion (5ml) was collected into EDITA containing tube for estimation of ESR by Westegren method. Second portion (5ml) was put in a plan tube, left to clot then centrifuged at 1600 rpm for 20 minutes and serum was separated and used for estimation of: C-reactive protein (CRP), (detected by latex agglutination slide test, using Omega Diagnostics, Scotland, UK). Positive test: agglutination will occur within 2 minutes, showing a CRP level equal or higher than 6 mg/l.[13] Rheumatoid factor (RF) was detected by latex agglutination slide test, using Omega Diagnostics, Scotland, and UK. Positive test: agglutination will occur within 2 minutes, showing a RF level equal or higher than 8 mg/l.[14] Anti-citrullinated protein antibodies (ACPA) was detected by ELISA immunoassay Kits (ORGENTEC Diagnostica GmbH) according to manufacturer instructions. Interleukin 23 (IL-23) was detected by ELISA immunoassay Kits from R&D Systems, Minneapolis, MN, USA. Catalogue number D2300B. Briefly, standards, samples and controls were pipetted into wells, pre-coated with a polyclonal antibody specific for the IL-23 p19 subunit and any IL-23 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for the IL-23 p40 subunit is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of IL-23 bound in the initial step. The color development is stopped and the intensity of the colour is measured and the microtiter plate was then read at 450nm wave length. The level of IL-23 was calculated from standard curve corresponding to the measured optical density. The results were expressed as pg/ml. Minimum detectable limit of IL-23 ranged from 2.7-16.3 pg/mL.

Statistical Methods

IBM SPSS statistics (V. 21.0, IBM Corp., USA, 2012) was used for data analysis. Data were expressed as Mean±SD for quantitative parametric measures in addition to Median Percentiles for quantitative non-parametric measures and both number and percentage for categorized data. The following tests were done: (1) Comparison between two independent mean groups for parametric data using Student t test. (2) Comparison between two independent groups for non-parametric data using Wilcoxon Rank Sum test. (3) Ranked Sperman correlation test to study the possible association between each two variables among each group for non-parameteric data. (4) Chi-square test to study the association between each 2 variables or comparison between 2 independent groups as regards the categorized data. The probability of error at 0.05 was considered significant; while at 0.01 and 0.001 were highly significant.

RESULTS

In this study, we prospectively enrolled 35 RA patients with mean age (46.5±10.5) and mean BMI (31±4.1); all are women and 15 age and BMI matched healthy women (44.6±10 and 30.9±3.8 respectively)

as control group. Demographic, clinical and laboratory data of RA patients and control were presented by table 1. Comparison between IL-23 in RA patients and healthy controls was shown in Table (2). There was significant elevation of IL-23 serum level in RA patients [43.6 (27.8-248.9)] compared to control group [32.1 (27.3-34.9)], P <0.05, (Figure 1). Correlations were done between IL23 and all data in RA patients. We found no correlations between IL-23 and activity in RA patients (DAS-28 score). Also, no correlations were found between IL 23 and clinical data of patients as duration of the disease, number of small or large joints affected (Table 3).

In order to further characterize the relationship between increased IL23 and activity in RA patients, we classify the RA patients into 4 groups according to DAS 28 score:

- **Group 1:** Include 10 patients in remission (DAS 28 score < 2.6). Their mean age was 43.6±8.6, mean BMI was 31.5±5.2 and the median duration of the disease was 5 years (ranging between 2-12 years).
- **Group 2:** Include 8 patients with low disease activity (DAS 28 score ranges between 2.6-3.2). Their mean age was 46.25±7.1, mean BMI was 31±5.1and the median duration of the disease was 9 years (ranging between (3-17) years).
- **Group 3:** Include 10 patients with moderate disease activity (DAS 28 score ranges between 3.2-5.1). Their mean age was 47.5±13.3, mean BMI was 31.4±3 and the median duration of the disease was 5 years (ranging between (1-11 years).
- **Group 4:** Include 7 patients with High disease activity (DAS 28 score > 5.1). Their mean age was 49.7±12.8, mean BMI was 30.1±3.5 and the median duration of the disease was 5 years (ranging between (1-15 years) (Table 4 and Figure 2).

The median of serum IL 23 increase gradually by increasing the DAS 28 score in group 1, 2, 3, and 4 [33.25 (27.8-51.5), 36.1(29-91), 40.9 (28.8-248.9), 52.1 (31.5-108.6) respectively]. However, this increased serum level did not reach a statistically significant differences when compared in the 4 groups (Table 5).

Table 1. Demographic, clinical and laboratory data of RA patients and control group.*

Variables	Patients N= 35	Control N=10
Age (years)	46.5±10.5	44.6 ± 10
BMI	31±4.1	30.9 ± 3.8
Duration of the disease (years)	5 (1-17)	
Number of small joints affected	4 (0-12)	
Number big joint affected	4 (1-8)	
DAS 28	3.5 ± 1.3	
Positive RF (%)	35 (100)	0
Positive CRP (%)	27 (77.1)	0
ESR	68 (28-150)	22 (13-30)
ACPA U/ml	37.1 (5.5-149)	

DAS 28 28-joint disease activity score; ***RF*** rheumatoid factor; ***CRP*** C-reactive protein;
ACPA Anti-citrullinated protein antibodies; IL-23: interleukin 23
*Results are expressed as mean ± standard deviation, median (range) or percent as appropriate.

Table 2. Comparison between IL-23 in RA patients and healthy controls.

Groups	IL- 23 (pg/ml)	P-value
Control group (N=15)	32.1(27.3-34.9)	0.012
RA patients (N=35)	43.6 (27.8-248.9)	

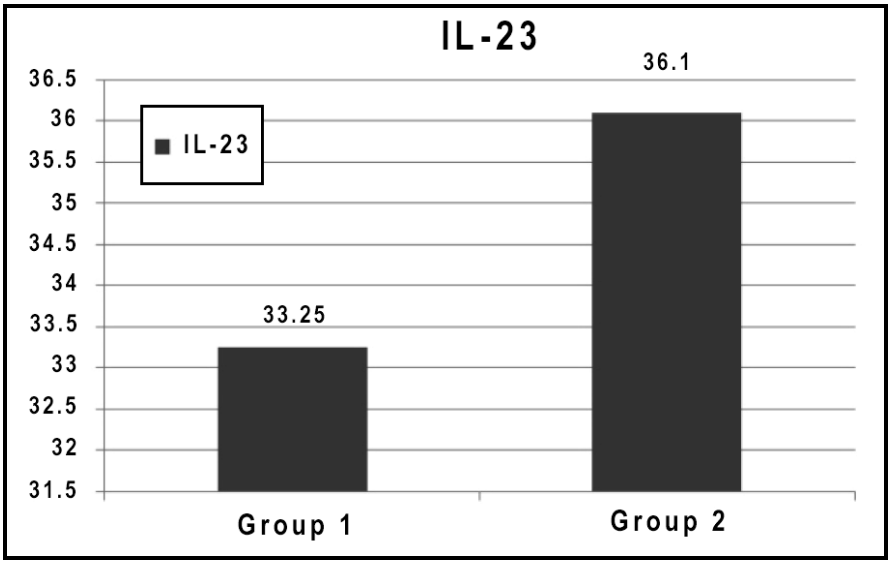

Figure 1. IL-23 level in RA patients and healthy controls.

Table 3. Correlation between serum IL-23 and duration of the disease, number of small and large joints affected, DAS(28) score, ESR and ACPA.

Variables	IL23	
	R	P
Duration of the disease	0.153	0.379
Number of small joints affected	0.117	0.501
Number big joint affected	0.157	0.369
DAS (28) score	0.109	0.534
ESR	0.069	0.694
ACPA	0.152	0.383

DAS 28 28-joint disease activity score; ***ACPA*** Anti-citrullinated protein antibodies; ***IL-23*** interleukin 23

Table 4. Demographic, clinical and laboratory data of RA patients classified according to DAS (28) score.

Variables	Group 1 N= 10	Group 2 N= 8	Group 3 N=10	Group 4 N=7
Age	43.6±8.6	46.25±7.1	47.5±13.3	49.7±12.8
BMI	31.5±5.2	31±5.1	31.4±3	30.1±3.5
Duration of the disease	5(2-12)	9 (3-17)	5 (1-11)	5 (1-15)
Number of Small Joints affected	2(0-7)	3 (0-4)	4 (0-6)	6 (2-12)
Number of Big joints affected	4(2-8)	4 (1-8)	5(2-8)	4 (2-8)
DAS (28) score	1.96±0.2	3.1±0.1	4.1±0.5	5.4±0.2
ESR	49±19.7	67±22.8	70.7±38.2	104.5±32.3
ACPA	32.3±18	36.2 (4.4)	51.76±25.6	92.4±48
IL-23	33.25 (27.8-51.5)	36.1(29- 91)	40.9 (28.8-248.9)	52.1 (31.5-108.6)

DAS 28 28-joint disease activity score; ***ACPA*** Anti-citrullinated protein antibodies; ***IL-23*** interleukin 23

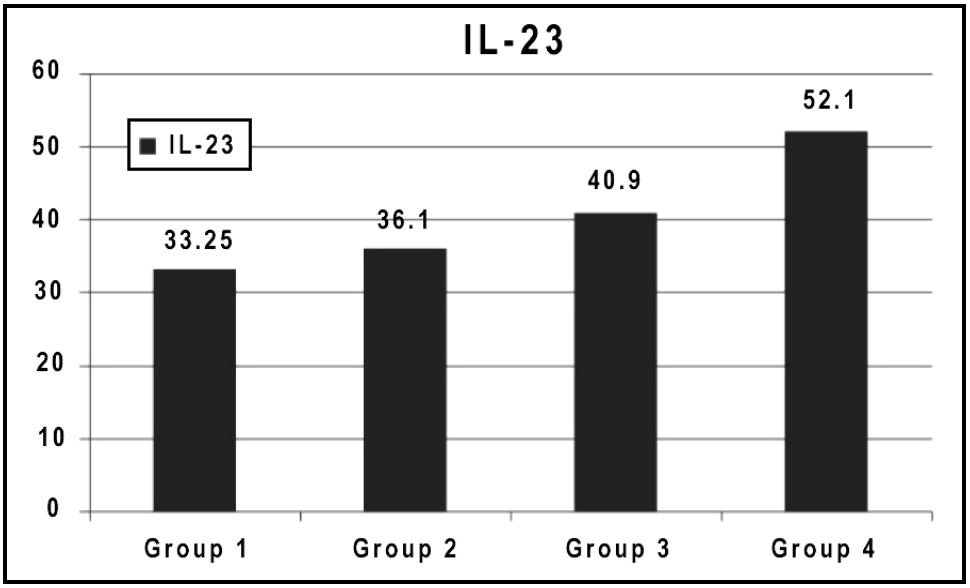

Figure 2. IL 23 serum level in RA patients classified according to DAS (28) score.

Table 5. Comparison between serum levels of IL-23 in RA patients classified according to DAS (28) score.

	Group 1 N=10	Group 2 N=8	Group 3 N=10	Group 4 N=7	P-value
Serum IL-23	33.25 (27.8-51.5)	36.1(29- 91)	40.9 (28.8-248.9)	52.1 (31.5-108.6)	> 0.05

DISCUSSION

RA is a systemic autoimmune disorder characterized by articular inflammation that eventually leading to joint destruction. The autoimmune response in RA is initiated by the activation of antigen-specific T cells.[15] Th17 cells are a distinct subset of T helper cells that play a role in the activation of the immune system by stimulating proinflammatory cytokine production via IL-17.[16] Results from studies using different arthritis models have demonstrated that IL-17-producing T-cells are the dominant cell type in the development of arthritis. IL-23 (a pro-inflammatory cytokine belonging to the IL-12 family) is essential for the differentiation of Th17 lymphocytes. In addition, a critical role of the IL-23/IL-17 axis in the progression to chronic destructive arthritis has been demonstrated.[17] This study was conducted to determine the role of IL23 in the pathogenesis of RA and its relation to various parameters of disease activity. Serum level of IL-23 was significantly elevated in RA patients [43.6 (27.8-248.9)] compared to control group [32.1 (27.3-34.9)] ($P < 0.05$). These results are consistent with the previous studies of Rasmussen and his colleague[18] that revealed significant increased plasma level of IL-23 in patients with early-stage RA compared to healthy volunteer controls. Also our results were consistence with the previous results of Guo et al. and Wang et al. that revealed significantly elevated serum IL23 in patients with RA compared to healthy controls.[19,20] When comparing the IL-23 between RA and osteoarthritis patients, the level of IL-23 was detected in a significantly higher proportion in RA patients than OA patients.[21] Our finding characterize a pathogenic role of IL 23 in RA patients and augmenting the previous data to support the nation that IL-23 may be a useful index for the diagnosis of RA. IL-23 is a heterodimeric cytokine, which is composed of the p40 subunit in common with IL-12, and with a unique p19 subunit. Kim et al. revealed that IL-23p19 is over-expressed in RA synovial fibroblasts and IL-17 appears to up-regulate the expression of IL-23p19 in RA synovial fibroblasts.[22] The concentration of IL-23p19 correlated with the concentration of IL-17

in synovial fluid and sera of RA patients. They concluded that upregulated IL-23p19 in synovial fluid might be involved in joint destruction in RA through interplay with IL-17. These results suggest that a disruption of interaction between IL-17 and IL-23p19 may provide a new therapeutic approach in the treatment of RA. In mice experimental arthritis, targeting IL-23p19 through a vaccination strategy is protective against joint destruction and inflammation.[23]

Our results also revealed no correlation between IL-23 and disease activity measured by DAS (28) score and in spite of gradual increase in the median level of serum IL-23 in RA patients with remission, low activity, moderate activity and severe activity, this increased serum level failed to achieve a significant difference. In consistent with our data, Chen et al. demonstrated significantly elevated serum IL-23 in active RA patients [24] and with Guo et al., who revealed positive correlation between serum IL-23 levels in the RA patients and the DAS28.[19] However, these contradictory results may be related to differences among the RA patients as regard to medication administered and the joint X-ray findings. IL-23 was significantly decreased, in parallel with the clinical remission in responders after anti-TNF-α therapy (etanercept).[24,25] The levels of IL-23 based on X-ray classification phase I, II, III, and IV were gradually elevated in RA patients.[19] We also observed no correlation between IL-23 and clinical parameters of arthritis as duration of the disease, number of small or large joints affected.

In conclusion, our study can implies that IL-23 cytokine may potentially play a role in the pathogenesis of RA and may be a useful index for the diagnosis of this disease. We also found that the increased level of IL-23 not correlated to disease activity measured by DAS 28 score. The sample size of this study was too small to establish a definitive conclusion. Further study is necessary to evaluate the pathogenic role of IL-23 in RA and to investigate the IL-23/IL-17 axis as a therapeutic target.

[Disclosure: Authors report no conflict of interest]

REFERENCES

1. Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. Lancet 2010, 376: 1094-1108.
2. Toussirot E. The IL23/Th17 pathway as a therapeutic target in chronic inflammatory diseases. Inflamm Allergy Drug Targets. 2012 Apr; 11(2): 159-68.
3. Chiricozzi A, Zhang S, Dattola A, et al.: New insights into the pathogenesis of cutaneous autoimmune disorders. J Biol Regul Homeost Agents. 2012 Apr-Jun; 26(2): 165-70.
4. Langrish CL, Chen Y, Blumenschein WM, et al.: IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. J Exp Med 2005; 201: 233-40.
5. Kirkham BW, Lassere MN, Edmonds JP, et al.: Synovial membrane cytokine expression is predictive of joint damage progression in rheumatoid arthritis: a two-year prospective study (the DAMAGE study cohort). Arthritis Rheum. 2006; 54(4): 1122-31.
6. Sato K, Suematsu A, Okamoto K, et al.: Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction. J Exp Med 2006; 203: 2673-2682.
7. Van Bezooijen RL, Van Der Wee-Pals L, Papapoulos SE, et al.: Interleukin 17 synergises with tumour necrosis factor alpha to induce cartilage destruction in vitro. Ann Rheum Dis. 2002; 61(10): 870-6.
8. Lubberts E, Koenders MI, van den Berg WB. Review: The role of T-cell interleukin-17 in conducting destructive arthritis: lessons from animal models.Arthritis Res Ther. 2005; 7(1): 29-37.
9. Stamp LK, Easson A, Pettersson L, et al.: Monocyte derived interleukin (IL)-23 is an important determinant of synovial IL-17A expression in rheumatoid arthritis. J Rheumatol. 2009; 36(11): 2403-8.
10. Paradowska-Gorycka A, Grzybowska-Kowalczyk A, Wojtecka-Lukasik E, et al. IL-23 in the pathogenesis of rheumatoid arthritis. Scand J Immunol. 2010; 71(3): 134-45.
11. Singh JA, Furst DE, Bharat A, et al.: Review 2012 update of the 2008 American College of Rheumatology recommendations for the use of disease-modifying antirheumatic drugs and biologic agents in the treatment of rheumatoid arthritis. Arthritis Care Res (Hoboken). 2012; 64(5):625-39.
12. Prevoo ML, van't Hof MA, Kuper HH, et al.: Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum. 1995; 38(1): 44-8.
13. Cecil RL, Nichols EE. Am. J. Med Sci., 181: 12 (1931)
14. Anderson HC, McCarthy M. Am. J. Med., 8: 445 (1950).
15. Paradowska A, Masliniski W, Grzybowska-Kowalczyk A, et al.: The function of interleukin 17 in the pathogenesis of rheumatoid arthritis. Arch Immunol Ther Exp (Warsz) 2007; 55: 329-334.
16. Bettelli E, Oukka M, Kuchroo VK. Review T(H)-17 cells in the circle of immunity and autoimmunity. Nat Immunol. 2007; 8(4):345-50.
17. Cornelissen F, van Hamburg JP, Lubberts E. The IL-12/IL-23 axis and its role in Th17 cell development, pathology and plasticity in arthritis. Curr Opin Investig Drugs. 2009; 10(5):452-62.

18. Rasmussen TK, Andersen T, Hvid M, et al.: Increased interleukin 21 (IL-21) and IL-23 are associated with increased disease activity and with radiographic status in patients with early rheumatoid arthritis. J Rheumatol. 2010; 37(10): 2014-20.
19. Guo YY, Wang NZ, Zhao S, et al.: Increased interleukin-23 is associated with increased disease activity in patients with rheumatoid arthritis. Chin Med J (Engl). 2013; 126(5): 850-4.
20. Wang T, Zhao Y, Liu X, et al.: Possible mechanisms of peripheral elevated CD8+ IL-17+ T cells in rheumatoid arthritis. Zhonghua Yi Xue Za Zhi. 2009 21; 89(7): 1885-8.
21. Kim J, Kang S, Kim J, et al.: Elevated levels of T helper 17 cells are associated with disease activity in patients with rheumatoid arthritis. Ann Lab Med. 2013; 33(1): 52-9.
22. Kim HR, Cho ML, Kim KW, et al. Up-regulation of IL-23p19 expression in rheumatoid arthritis synovial fibroblasts by IL-17 through PI3-kinase-, NF-kappaB- and p38 MAPK-dependent signalling pathways. Rheumatology (Oxford). 2007; 46(1): 57-64.
23. Ratsimandresey RA, Duvallet E, Assier E, et al.: Active immunization against IL-23p19 improves experimental arthritis. Vaccine. 2011;29(50):9329-36.
24. Chen DY, Chen YM, Chen HH, et al.: Increasing levels of circulating Th17 cells and interleukin-17 in rheumatoid arthritis patients with an inadequate response to anti-TNF-α therapy. Arthritis Res Ther. 2011 Jul 30; 13(4): R126.
25. Kageyama Y, Ichikawa T, Nagafusa T, et al.: Etanercept reduces the serum levels of interleukin-23 and macrophage inflammatory protein-3 alpha in patients with rheumatoid arthritis. Rheumatol Int. 2007; 28: 137-143.